# Analytical Chemistry of Carbohydrates

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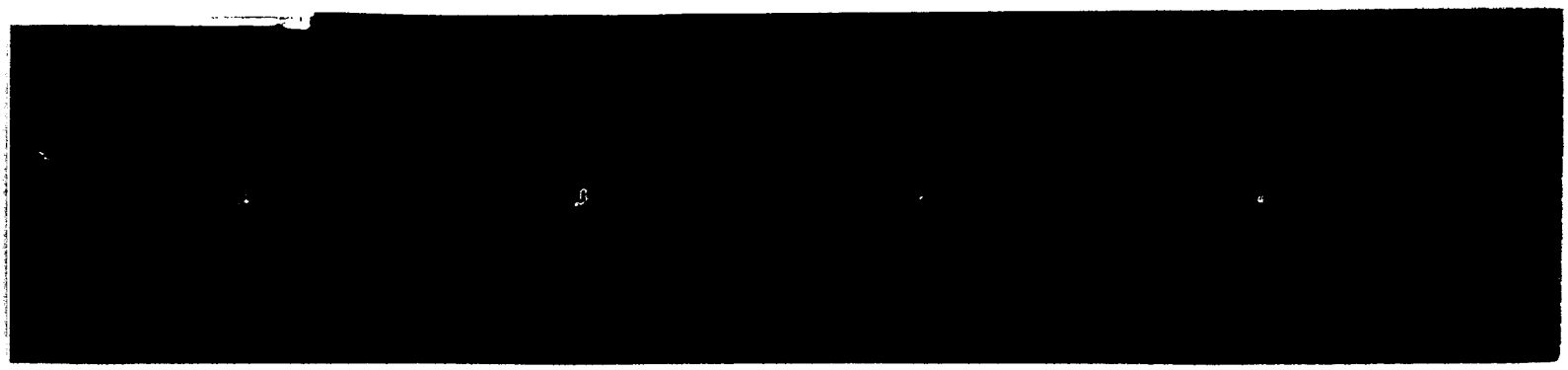


anhydrous sodium sulfate or anhydrous calcium chloride, and evaporate the organic phase again to dryness. Then, add 200 µL dichloromethane containing tetra-*O*-acetyl-*meso*-erythritol as internal standard for subsequent GC–MS studies.

*(b) Reaction with TMS-O-Mesylate–$BF_3 \cdot Et_2O$*[85,86]

*Reagent A:* a mixture of *O*-trimethylsilyl methanesulfonate (TMS-*O*-mesylate), $BF_3 \cdot Et_2O$ and triethylsilane (TES): 5 equiv. TMS-*O*-mesylate, 1 equiv. $BF_3 \cdot Et_2O$ and 5 equiv. TES per 1 equiv. glycosidic bond prepared immediately before use.

*Reagent B:* a mixture of acetic anhydride–trifluoroacetic acid 10:1 v/v.

Dissolve 0.5–2.0 mg of the permethylated polysaccharide, in a 2 mL vial, under nitrogen in 200 µL dry dichloromethane and add reagent A. Stir for 3–14 h at room temperature, add 30 µL of reagent B and stir for another 15 min at 50 °C. Isolate the acetylated compounds according to the procedure above (a).

Figure 3 shows the GC of the partially methylated anhydroalditol acetates by the reductive cleavage (using the TES–TMS-*O*-mesylate–$BF_3 \cdot Et_2O$ reagent) of methylated agarose[86].

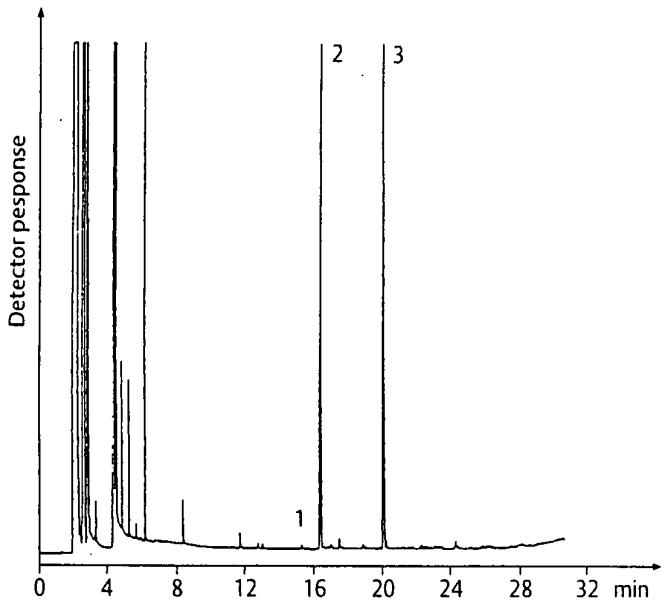



**Figure 3.** Gas–Liquid Chromatogram of Partially Methylated Anhydroalditol Acetates Derived from TES–TMS-*O*-Mesylate–$BF_3 \cdot Et_2O$ Cleavage of Per-*O*-methylated Agarose
*Column*: JW-DB-5 fused silica column, L: 30 m, i.d.: 0.25 mm; *temperature/time*: 70 °C, 3 min; 70–120 °C/20 °C $min^{-1}$; 120 °C, 5 min; 120–300 °C/5 °C $min^{-1}$
1: 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-galactitol, 2: 4-*O*-acetyl-1,5:3,6-dianhydro-2-*O*-methyl-L-galactitol, 3: 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl-D-galactitol (Kiwitt-Haschemie, Heims, Steinhart and Mischnik[86]; with permission)



## 3.3.3. Other Methods for the Determination of Polysaccharide Structures

Other methods for the elucidation of the chemical structures of polysaccharides must determine (a) the stereochemical structure of the single units including their linkages and (b) the sequences of the single units in the chains of the polysaccharide molecules.

In the first case, a specific oxidation reaction with periodate and sodium borohydride ("Smith degradation") can be used in addition to the methylation procedure (Section 3.3.2.).

### 3.3.3.1. Cleavage of the Polysaccharide with Periodate (Smith Degradation)

The reaction of vicinal polyhydroxy compounds with periodate ions, the so-called "Malaprade reaction" (see Section 1.3.1.), leads to cleavage of the C–C bond between two vicinal primary or secondary H–C–OH groups yielding two aldehyde groups, which can be reduced in aqueous solution with sodium borohydride to the corresponding alcohols. Application of this reaction to polysaccharides depends on the position of the glycosidic linkages between the single units and whether the single unit itself has, for example, a furanose or pyranose structure, as demonstrated in the following schemes[87–89] (see page 328).

Different practical procedures exist for carrying out this reaction[90–92]; the micromethod developed for the elucidation of the structures of soluble bacterial polysaccharides is described here in detail[92].

**Procedure**

*Reagent A:* 0.1 M aqueous sodium acetate buffer pH 3.9.

*Reagent B:* 0.2 M aqueous solution of sodium metaperiodate.

*Reagent C:* ethylene glycol, pure.

*Reagent D:* sodium borohydride pure.

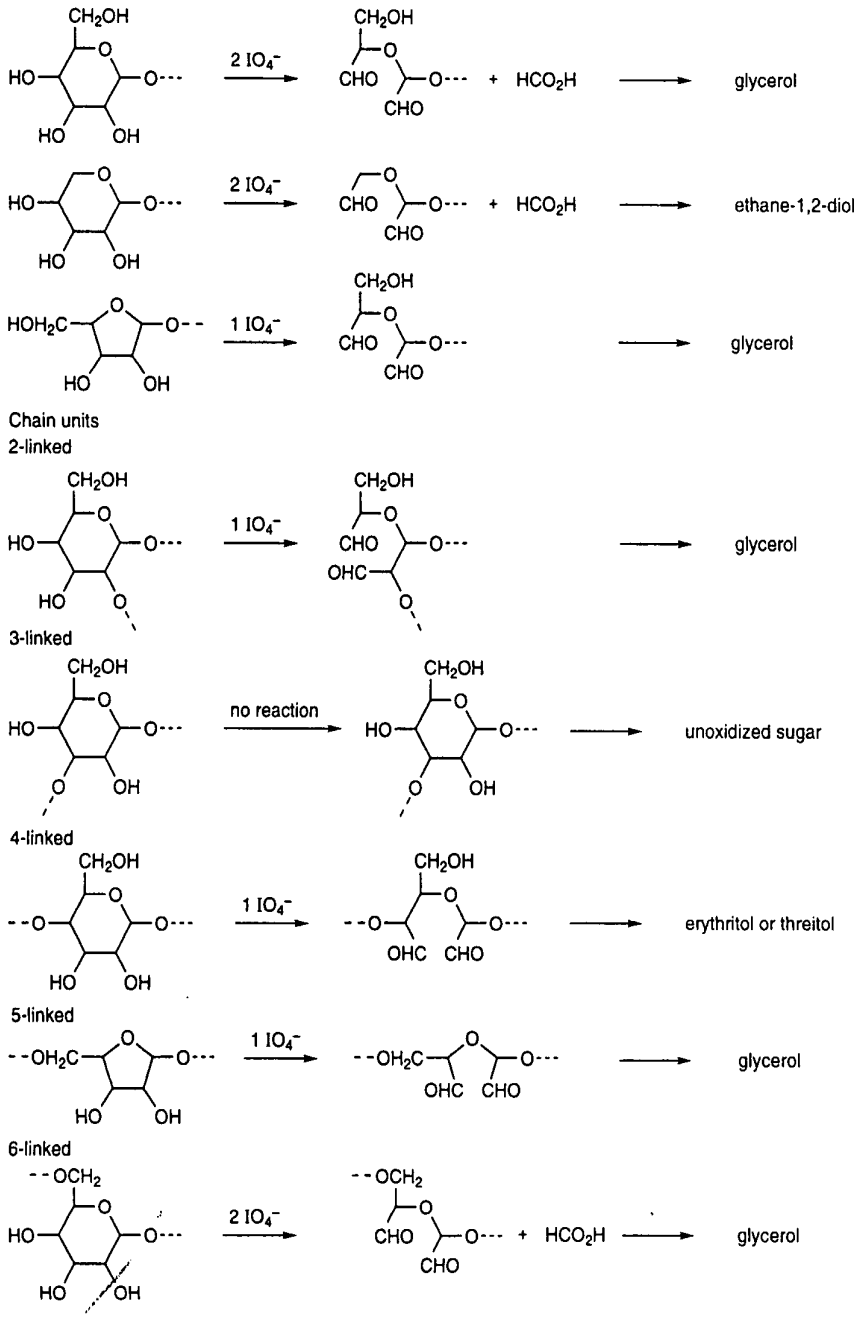
*Reagent E:* 50% aqueous acetic acid.

Dissolve approximately 30 mg of the polysaccharide in a small amount of reagent A and add 5 mL of reagent B. Keep the solution in the dark at 4 °C for 120 h, then add 1 mL of reagent C to destroy the excess of reagent and dialyze the solution against tap water overnight. Concentrate the solution of the oxidized polysaccharide in vacuo to approx. 50 mL, add 300 mg of reagent D and keep the solution for 8 h at room temperature. Decompose reagent D by addition of reagent E and dialyze the solution overnight; concentrate the solution or lyophilize it to obtain a dry product.

The obtained altered polysaccharide can be treated in different ways:

- Hydrolysis with weak acid (0.05–0.1 M sulfuric acid) for cleaving only the acetal groups, liberating low molecular sugar alcohols like glycerol, erythritol, etc.
- Hydrolysis with medium strength acids like 0.25 M sulfuric acid giving lower polyalcohols as well as nonaltered sugar units.
- Methylation of the periodate-treated polysaccharide followed by hydrolysis.

The low molecular weight degradation products are analyzed qualitatively and quantitatively, preferably by GC–MS, as their *O*-acetyl derivatives (see Section 2.1.4.). In several cases, polysaccharides show so-called "nonideal behavior" which can be divided into two groups, namely, (a) overoxidation and (b) incomplete oxidation. Overoxidation[93,94] is encountered when the reaction with periodate yields either tartronaldehyde derivatives or tartronic acid–half aldehyde derivatives (e.g., from hexafuranosides or hexuronic acid end groups). Such nonspecific oxidation reactions are limited when the reaction is carried out at a pH range of 2.2–4.0, but preferably at pH 3.6.




Reactions of Periodate with Differently Linked Sugar Units



Incomplete oxidation has two main causes:

(a) Hemiacetal formation between aldehyde fragments in oxidatively cleaved residues and hydroxyl groups in adjacent but not yet oxidized residues; the latter units are then protected against further oxidation[95]. The highest degree of such incomplete oxidation occurs in 4-linked polysaccharides without primary hydroxyl groups at C-6[96].

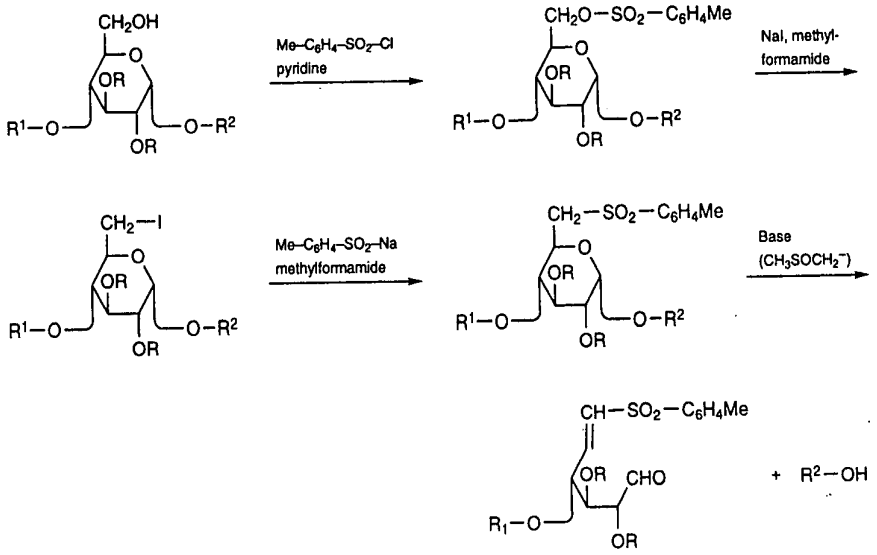
(b) Hydrogen bonds between one of the pair of hydroxyl groups resp. carboxyl groups and a suitably disposed acetamino group on a neighboring sugar residue[97,98].

### 3.3.3.2. Further Reactions

These are applied for particular purposes, especially for the determination of the frequencies of the single units in a polysaccharide chain. They are only briefly mentioned here.

#### (a) Base-Catalyzed β-Fragmentation

This can be initiated by strongly electron-withdrawing functional groups leading to the cleavage of the glycosidic linkage[99]. The best known is the degradation of esterified polyuronic acid units containing polysaccharides, such as highly esterified pectins[100,101] or 3-*O*-polyglycan laminaran[101–103] (see pages 323, 324). Another modification is the conversion of the free primary hydroxyl group of a methylated polysaccharide unit into a sulfone group with 4-toluenesulfonyl chloride (Ts–Cl) according to the following scheme[104–106].




Base-Catalyzed β-Elimination of 6-Toluene Sulfone Units of a Methylated Polysaccharide

The methylated polysaccharide possessing a free primary alcohol group (e.g., obtained by reduction of a methylated uronic acid containing polysaccharide with lithium aluminum hydride) is treated with 4-tolunenesulfonyl chloride in pyridine yielding the respective tosyl ester. These units are converted with sodium iodide in methylformamide into the 6-deoxy-6-iodo residues. Further reaction with sodium 4-toluenesulfinate in methylformamide introduces the toluene sulfone group into the 6-position of these units of the methylated polysaccharides. Treatment of the sulfone units with base cleaves the glycosidic bond to the next unit. The residue R–OH can be identified by further methylation.

The latter reaction has been used for the elucidation of the structures of bacterial polysaccharides[104, 105] as well as galactomannans[106].

**(b) Partial Depolymerization in an Aqueous Medium[107,108]**

The method of partial depolymerization results in a mixture of different oligosaccharides. After separation, the structure of each compound is determined by methods described in the previous chapters and additionally by $^{13}C$ and $^{1}H$ NMR and GC–MS. The structure of the polysaccharide can be elucidated by piecing the structure of the single oligosaccharides together like a puzzle.

As remarked in Section 3.3.1., differences in the cleavage rates of the glycosidic linkages exist among sugar units of the same type and ring sizes, but linked at different positions as well as among the single units themselves[107]. α-D-Glycopyranosyl linkages are generally more easily hydrolyzed than β-D-bonds; linkages of the (1→6)-type are more resistant than those at other positions and furanoside rings hydrolyze $10–10^3$ faster than pyranoside rings. Glycosiduronic acid[109] and particularly 2-amino-2-deoxy glycosidic linkages are resistant towards acid hydrolysis. The property of the latter compounds is caused by the inductive effect of the totally protonated 2-amino group which prevents further protonation of the glycosidic bond[110]. Acetylation of such amino groups diminishes the resistance against hydrolysis. In native polysaccharides these amino sugar units are acetylated in most cases. After deacetylation, either by hydrazinolysis[111] or treatment with sodium hydroxide in DMSO[112], the resulting 2-amino-2-deoxy sugar linkages are almost resistant to hydrolysis and such amino sugar disaccharides can be isolated as products of partial hydrolysis.

**(c) Partial Depolymerization in Nonaqueous Medium**

Acetolysis: polysaccharides are cleaved with a mixture of acetic anhydride and sulfuric acid yielding acetylated oligosaccharides, which can be separated and identified. The rates of cleavage depend on the nature of the glycosidic linkages and are different from those in an aqueous medium. For example, the (1→6)-linkages are more easily cleaved than the others[113] (in aqueous medium it is inverted). The terminal 6-deoxyhexapyranosyl bonds (e.g., those of L-rhamnose[115] or L-fucose[114]) are remarkably stable. The sialic acid linkages also remain intact during this treatment[116], in contrast to that in an aqueous acid medium where they are very labile.

It must be remarked further, that anomerization occurs during acetolysis particularly at (1–6)-linkages; the energetically mote stable α-anomers are formed also from β-linkages of the original polysaccharides[117].

Trifluoroacetolysis: this method uses mixtures of trifluoroacetic anhydride and trifluoroacetic acid and is frequently applied in the field of glycoconjugates[118–120].



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